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# MOLECULAR MECHANISMS OF BONE RESORPTION IN PERIODONTITIS

KUMULATIVE HABILITATIONSSCHRIFT

ZUR ERLANGUNG DER VENIA LEGENDI
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GEORGIOS N. BELIBASAKIS, DDS, MSC, PHD

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**The following original publications are submitted as a "Kumulative Habilitationsschrift" thesis, for a post-doctoral lecture qualification:**

***Paper 1***

Bostanci N, Ilgenli T, Emingil G, Afacan B, Han B, Töz H, Berdeli A, Atilla G, McKay I J, Hughes F J, **Belibasakis G N**. Differential expression of RANKL and OPG mRNA in periodontal diseases. *J Periodontal Res* 2007; 42: 287-293

***Paper 2***

**Belibasakis G N**, Bostanci N, Hashim A, Johansson A, Aduse-Opoku J, Curtis MA, Hughes FJ. Regulation of RANKL and OPG expression in gingival fibroblasts and periodontal ligament cells by *Porphyromonas gingivalis*: a putative role of the Arg-gingipains. *Microb Pathog* 2007; 43: 46-53

***Paper 3***

**Belibasakis G N**, Reddi D, Bostanci N. *Porphyromonas gingivalis* induces RANKL in T-cells. *Inflammation* 2011; 34: 133-138

***Paper 4***

**Belibasakis G N**, Meier A, Guggenheim B, Bostanci N. The RANKL-OPG system is differentially regulated by supragingival and subgingival biofilm supernatants. *Cytokine* 2011; 55: 98-103

# MOLECULAR MECHANISMS OF BONE RESORPTION IN PERIODONTITIS

## *BACKGROUND AND AIM*

Periodontal diseases are a group of highly prevalent oral infections that cause the inflammatory destruction of the tooth-supporting (periodontal) tissues. The inflammation can be restricted to the gingival tissue, clinically manifesting as gingivitis, or expand deeper to affect the tooth-supporting alveolar bone and periodontal ligament, manifesting as periodontitis. If left untreated, periodontitis may eventually lead to tooth loss. Etiologically, periodontal diseases are caused by indigenous oral bacteria, which attach on the tooth surface and grow as polymicrobial "biofilm" communities. Within biofilms, individual bacteria benefit from each other and develop strategies that prolong their survival in the host [1]. *Porphyromonas gingivalis* is a Gram negative anaerobe, typically present in subgingival biofilms from sites with periodontitis, particularly the chronic form. Due to its virulence factors, such as the cysteine proteinases (gingipains), *P. gingivalis* can deregulate the innate immune responses in favour of its survival within the host environment [2]. The host elicits a local inflammatory response against biofilms or their released bacterial products, which is meant to be protective. However, if this response becomes excessive, it could lead to damage of the supporting alveolar bone, resulting in periodontitis.

Bone resorption is regulated by the interplay of Receptor Activator of NF-kB Ligand (RANKL) and osteoprotegerin (OPG), two molecules belonging to the tumor necrosis factor ligand and receptor families, respectively [3]. RANKL, produced by various cells, stimulates its cognate RANK receptor on pre-osteoclasts and subsequently their differentiation into multi-nucleated osteoclasts, which will resorb bone. On the contrary, OPG inhibits the action of RANKL by binding to it, thus preventing osteoclast differentiation and bone resorption. A local increase of the RANKL/OPG ratio is observed at sites exhibiting enhanced bone resorption [4].

Since the RANKL-OPG system is crucial for controlling bone resorption, imbalances in its expression may cause a switch from a physiological to a pathological state at different sites of the human skeleton. Hence, the aim of these studies was to evaluate how this system could be involved in the pathogenesis of human periodontitis, by investigating clinical and mechanistic aspects of its regulation.

### *METHODS*

This work includes a clinical and an *in vitro* component, both ultimately studying RANKL and OPG gene expression in the two different experimental platforms. In the clinical part (Paper 1), gingival biopsies were obtained from periodontally healthy and diseased sites. The levels or RANKL and OPG mRNA expression in these samples was assessed by quantitative real-time Polymerase Chain Reaction (qPCR). The *in vitro* experimentations focused on the potential biological mechanisms of RANKL and OPG regulation. In two of the studies (Paper 2 and Paper 3), *P. gingivalis* products were added to host cell cultures, including gingival fibroblasts (GF), periodontal ligament cells and T-cells. RANKL and OPG mRNA expression in these *P. gingivalis*-challenged cells was investigated by qPCR. The relative effects of *P. gingivalis* gingipains, were evaluated by using genetically modified strains lacking the capacity to produce these virulence factors. The latest study (Paper 4) investigated accordingly the effects of polymicrobial biofilms on the RANKL-OPG system. GF cultures were challenged with secreted products of an *in vitro* supragingival biofilm model consisting of 6 species, or a subgingival biofilm model of 10 species, including *P. gingivalis*. The RANKL and OPG mRNA expressions were investigated by qPCR.

### *FINDINGS AND DISCUSSION*

The relevance of the RANKL-OPG system in human periodontitis was confirmed in the cross-sectional clinical study. RANKL was not expressed in healthy gingival tissues, but OPG was constantly expressed denoting its protective role from bone resorption. This pattern was similar in gingivitis, indicating that despite the on-going inflammation, the bone resorption mechanism is still not activated in this form of the disease. However, a limited number of sites with gingivitis also expressed RANKL, which could represent sites of on-going switch to periodontitis. To this extent, RANKL expression levels were increased in periodontitis, whereas OPG levels were significantly lower than in health or gingivitis. These differential expressions reflected an increased RANKL/OPG ratio in periodontitis, compared to health or gingivitis, confirming the association of this measure to enhanced bone destruction.

The mechanisms of RANKL-OPG regulation were investigated in more depth using *in vitro* experimental systems. The major periodontal pathogen *P. gingivalis* induced RANKL and partly reduced OPG expression in GF and PDL cells. This resulted in an increased RANKL/OPG ratio, which denotes an enhanced capacity by the cells to stimulate bone resorption. The *P. gingivalis* virulence factor responsible

for the induction of RANKL is potentially a protein which could be functionally associated with the gingipains, but a non-proteinaceous component is likely to be responsible for OPG reduction. *P. gingivalis* also induced RANKL in T-cells, which, however, were not able to produce OPG. These *in vitro* observations are in agreement the potential of *P. gingivalis* to act as a periodontal pathogen [2], and match the clinical findings, where the switch from health to periodontitis is associated with increased RANKL and decreased OPG expression.

As periodontitis is an infection of polymicrobial nature, it is rational to consider the effects of multi-species biofilms, in the regulation of the RANKL-OPG system. In the present experimental system, an *in vitro* supragingival and a subgingival biofilm model both caused enhancement of the RANKL/OPG expression ratio in GF, albeit the latter was considerably more potent in this respect. These findings provide a molecular proof of the clinical concept that subgingival biofilms are of higher virulence for the development of periodontitis [5].

### *CONCLUSION AND SIGNIFICANCE*

The molecular signature of periodontitis is characterised by increased RANKL and decreased OPG expression, resulting in an increased RANKL/OPG ratio, as in other bone destructive diseases, like rheumatoid arthritis or osteoporosis [4]. Putative periodontal pathogens associated with subgingival biofilms, such as *P. gingivalis*, are likely to regulate this system in favour of bone destruction, which is a hallmark of periodontitis. Understanding the role and regulation of the RANKL-OPG system in periodontitis may provide insights for development of molecular diagnostic or therapeutic targets for the disease, for instance by blocking RANKL [6], or by eradicating the stimulating microbial factors.

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